Form PTO 1390 (REV 5-93) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED / ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		P50800 U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5) 09/720965
INTERNATIONAL APPLICATION NO. PCT/US99/15366	INTERNATIONAL FILING DATE 07 July 1999	PRIORITY DATE CLAIMED 07 July 1998
TITLE OF INVENTION NOVEL FLUORESCENT LANTHANIDE CHELATES		
APPLICANT(S) FOR DO/EO/US George Wai-Kin CHAN and Robert Philip HERTZBERG		

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1 [x] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. [x] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. [x] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. [x] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] has been transmitted by the International Bureau.
   c. [x] is not required, as the application was filed in the United States Receiving Office (RO/US).

6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.

8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S. C. 371(c)(3)).

9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. [x] An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.

12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included.

13. [ ] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
    [x] Please amend the specification by inserting before the first line the sentence: This is a 371 of International Application PCT/US99/15366, filed 7 July 1999, which claims benefit from the following Provisional Application No.: 60/091,944, filed 7 July 1998.

14. [ ] A substitute specification.

15. [ ] A change of power of attorney and/or address letter.

16. [ ] Other items or information:

US APPLICATION NO. (if known, see 37 CFR 1.50) 09/720965	INTERNATIONAL APPLICATION NO. PCT/US99/15366	ATTORNEYS DOCKET NO. P50800

17. [X] The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 C.F.R. 1.492(a)(1)-(5)):		
Search Report has been prepared by the EPO or JPO **$860.00**		
International Preliminary Examination Fee paid to USPTO (37 CFR 1.482) **$690.00**		
No International Preliminary Examination Fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) **$710.00**		
Neither International Preliminary Examination Fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....**$1,000.00**		
International Preliminary Examination Fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4).....**$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	**$690.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	**$0.00**	

Claims	Number Filed	Number Extra	Rate		
Total claims	**10 - 20 =**	**0**	**0 x $18.00**	**$0.00**	
Independent claims	**3 - 3 =**	**0**	**0 x $80.00**	**$0.00**	
Multiple dependent claims (if applicable)			**+ $270.00**	**$0.00**	
			TOTAL OF ABOVE CALCULATIONS =	**$690.00**	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				**$**	
			SUBTOTAL =	**$690.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)) +				**$**	
			TOTAL NATIONAL FEE =	**$690.00**	
				Amount to be refunded	**$**
				charged	**$**

a. ☐ A check in the amount of $____ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. 19-2570 in the amount of **$690.00** to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 19-2570 . A duplicate copy of this sheet is enclosed.

d. ☒ General Authorization to charge any and all fees under 37 CFR 1.16 or 1.17, including petitions for extension of time relating to this application (37 CFR 1.136 (a)(3)).

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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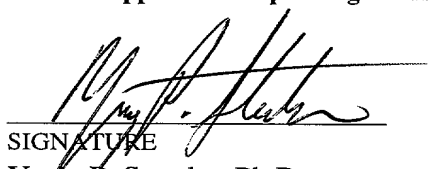


SIGNATURE
Yuriy P. Stercho, Ph.D.
NAME
33,797
REGISTRATION NO.

NOVEL FLUORESCENT LANTHANIDE CHELATES

FIELD OF INVENTION

The present invention relates to the identification and preparation of organic agents that can complex lanthanide cations. In particular, this invention relates to complexing agents which contain novel photosensitizers and can produce long-lived fluorescence for use in bioaffinity assays, especially HTRF (homogeneous time-resolved fluorescence) assays.

BACKGROUND OF THE INVENTION

A wide variety of bioassays are used in the pharmaceutical industry to identify drug development candidate compounds. Recent advances in the identification of pharmaceutical targets, together with the vastly increased output of new compounds using techniques such as combinatorial chemistry have created a need to increase bioassay throughput (number of samples measured per unit time) drastically to meet discovery objectives. Robotics, miniaturization and homogeneous assay formats have all been incorporated into high throughput screening (HTS) assays to increase throughput. Ideally, an analytical technique suitable for both miniaturization and homogeneous assay formats must provide maximal detection sensitivity and interaction *in situ*, while requiring only minimal assay time and liquid handling (e.g., separation and filtration). Present analytical techniques, such as those which use radiolabels, are unsatisfactory for HTS use because they lack sensitivity, require large sample size and manual liquid handling.

Compared to traditional radiolabels, fluorescent labels have more desirable lifetime, solubility and sensitivity properties for use in HTS assays. The unique lifetime properties of fluorescent labels also meet the needs of fluorescence polarization (FP) and fluorescence correlation spectroscopy (FCS) in the investigation of slow rotational and translational changes in macromolecules.

Traditional fluorescent labels such as organic dyes, e.g., fluoresceins and rhodamines, have long been employed as bioanalytical tools in immunoassays. More recently, lanthanide chelates have been developed as fluorescence agents for use in the bioassay field. These lanthanide chelates have been reviewed. *See* Dickson, J. Photochemistry and Photobiology, **27** (1995) 3-19; and Mathis, J. Clinical Ligand Assay **20** (1997) 141-145.

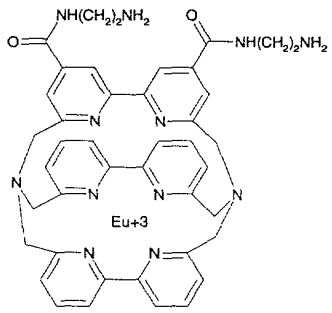
The lanthanide chelates are capable of producing long-lived and long wavelength fluorescent emissions upon excitation. In time-delay measurements, they have demonstrated clear advantages over conventional fluorescent labels, in particular less quenching and background interference, while exhibiting increased detection sensitivity. In addition to these advantages, many lanthanide chelates have demonstrated superior solubility properties and are able to efficiently transfer energy from their excited states to neighboring acceptor molecules. These advantages render lanthanide chelates ideal agents for HTRF use, especially for developing high-throughput automated and miniaturized binding assays, inncluding immunoassays, DNA hybridization assays, receptor binding assays, enzyme assays, cell-based assays, immunocytochemcial or immunohistochemical assays.

A number of lanthanide (e.g. terbium, europium) complexes are known, but only three classes of lanthanide chelates, exemplified by the compounds shown in Table I below, are considered to be useful in HTRF:

Table I

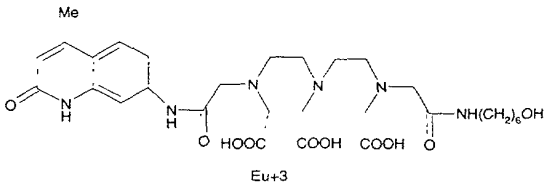
Cryptates (Packard)

bipyridine type; i.e.



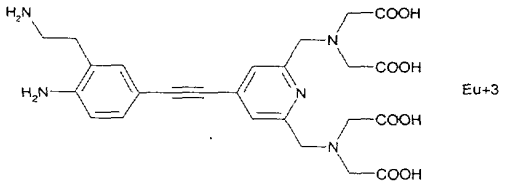
DTPA Chelates (Berkeley)

diethylenetriamine-pentaacetic acid type; i.e.



PMDA Chelates (Wallac)

pyridylmethylamine-diacetic acid type; i.e.



These chelates have been described as having chemical stability, long-lived fluorescence (greater than 0.1 ms lifetime) after bioconjugation and significant energy-transfer in specific bioaffinity assays. US5162508, issued to Lehn, et al. on November 10, 1992 discloses bipyridine cryptates. Polycarboxylate chelators with TEKES type photosensitizers (EP 0203047 A1) and terpyridine type photosensitizers (EP 0649020 A1) are known. International Publication No. WO 96/00901 of Selvin et al., having an International Publication Date of January 11, 1996, discloses diethylenetriaminepentaacetic acid (DTPA) chelates which used carbostyril as sensitizer. Bailey, et al., Analyst, **109**, (1984) 1449; Ando, et al. Biochim. Biophys. Acta, **1102**, (1992) 186; and Heyduk et al., Anal. Biochemistry, **248**, (1997) 216 also describe DTPA lanthanide chelates which contain different sensitizers. Additional DTPA chelates with other sensitizers and other tracer metals are known for diagnostic or imaging use (e.g., EP 0450742 A1).

The lanthanide chelates provided by the present invention include novel sensitizers which differ from carbostyril and other known chelates. More specifically, these novel sensitizers impart onto the present chelates advantageous physicochemical properties pertaining to excitation wavelength, lifetime, quantum yield, quenching effect, complex stability, photostability, solubility, charge, nonspecific protein interaction, biocoupling efficiency and ease of preparation. Such advantages are desirable to provide a diversity of novel fluorescent probes for use in, and development of, HTRF assays.

SUMMARY OF THE INVENTION

An object of the present invention is to provide novel lanthanide chelate compounds, and a method for using such compounds in fluorescence detection-based techniques or bioassays.

Accordingly, in the first aspect, this invention provides a compound according to Formula I.

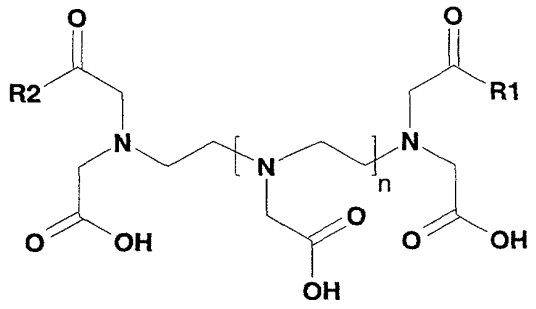
In still another aspect, this invention provides a method for using the compounds of Formula I in fluorescence detection-based techniques or bioassays.

In yet another aspect, this invention provides a kit for fluorescence detection-based techniques or bioassays which use the compounds of Formula I as the basis for signal detection and measurement.

DETAILED DESCRIPTION OF THE INVENTION

Each compound of the present invention comprises four functional parts: a lanthanide metal cation (e.g. Tb III, Eu III, Sm III, Dy III), a chelator for the lanthanide metal, a photosensitizer for photoexcitation and energy transfer, and a linker for bioconjugation to the target biomolecule, that is, the biomolecule being measured using a fluorescence detection -based spectroscopic technique or bioassay.

The present invention provides compounds of Formula I:

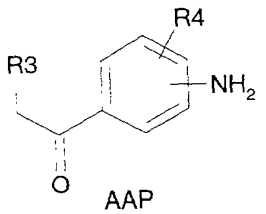


I

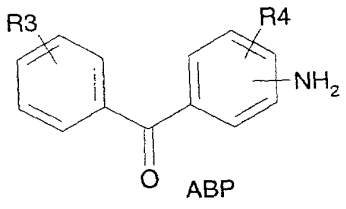
wherein:

$[\backslash N\Lambda]_n$ is a chelator selected from the group consisting of: diethylenetriaminepentaacetic acid (DTPA) (n = 1) or triethylenetetraaminehexaacetic acid (TTHA) (n =2) or a polyaminocarboxylate derivative of DTPA or TTHA, preferably DTPA, which chelates a lanthanide metal cation, preferably selected from the group consisting of: Tb III, Eu III, Sm III, and Dy III.

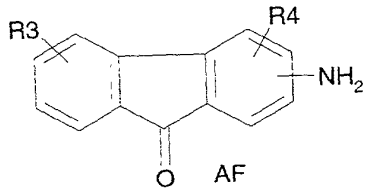
The sensitizer R1 is usually related to an aromatic or heteroaromatic amine whose chromophore plays a vital role in excitation and energy transfer. Superior sensitizers usually have highly conjugated systems and an added capacity for lanthanide complexation. We have found several sensitizers, belonging to two structural classes- phenones and quinolines - that provide highly fluorescent compounds of Formula I. R1 is more preferably selected from the following group: aminoacetophenones (AAP), aminobenzophenones (ABP), aminofluorenones (AF), aminoxantones (AX), amino-azaxanthones (AAX), aminoanthraquinones (AAQ), and aminoacridones (AAC):



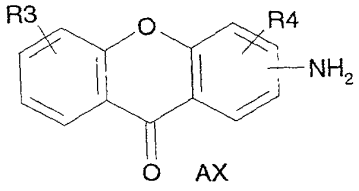
AAP



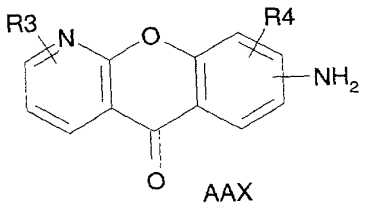
ABP



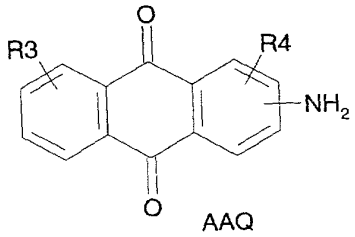
AF



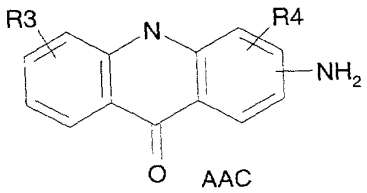
AX



AAX



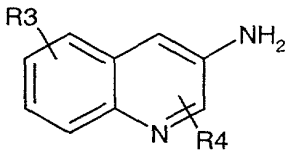
AAQ



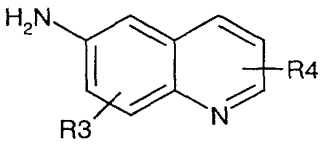
AAC

wherein for each nucleus, the amino group NH_2 may be attached at one of any possible positions on the phenyl ring. The point of amide attachment to the chelator $[\backslash N/\backslash]_n$ in Formula I may similarly be attached at one of any possible positions on the phenyl ring. R3 and R4 are independently selected from the group consisting of: H, OH, NH_2, $COCH_3$, COPh, OPh, NHPh, CN, NO_2, CO_2H, CO_2CH_3, I, Br and Cl.

Sensitizers of the present invention belonging to the quinoline class can be further categorized into 3- aminoquinolines (3AQ), and 6-aminoquinolines (6AQ). Preferably in the quinoline compounds of the present invention, R1 is selected from the group consisting of:



3AQ



6AQ

wherein R3 and R4 are as defined herein above.

The linker R2 is an amine or other moiety having a functional group that can bioconjugate or can be derivatized to couple with biomolecules. In a preferred embodiment of the present invention, R2 is selected from the group consisting of: OH, $NH(CH_2)_nOH$, $NH(CH_2)_nNH_2$, $NH(CH_2)_nPhNH_2$, $NH(CH_2)_nPhOH$, $NHCH(CO_2H)CH_2PhNH_2$,, $NH(CH_2)_nPhNCS$; wherein n is 1-12. The present invention also contemplates the use of other linkers known in the art for coupling.

Particularly preferred compounds of the present invention include the DTPA chelates listed in Table II below:

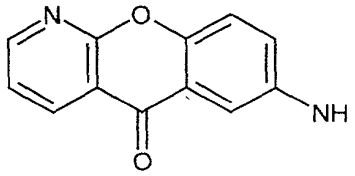
Table II

Formula	R1	R2	Lifetime, msec	
			Lanthanide	
			Eu	Tb
I	3AAP	-	0.59	1.73
I	3AQ	-	0.59	
I	6AQ	-	0.60	
I	4ABP	-	0.60	1.03
I	3AAP	4APEA	0.50	1.62
I	3AAP	4APEA-ITC	0.62	1.65
I	3AAP	4APA	0.60	1.70
I	3AQ	4APEA		
I	3AQ	CAD		
I	6AQ	4APEA		
I	6AQ	CAD	0.58	
I	4ABP	4APEA	0.43	0.73
I	4ABP	CAD	0.59	0.82

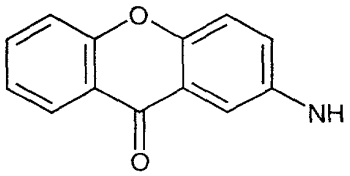
Abbreviations:

3AAP:	4-aminoacetophenone
3AQ:	3-aminoquinoline
6AQ:	6-aminoquinoline
4ABP:	4-aminobenzophenone
4APEA:	4-aminophenethylamine
4APEA-ITC:	4-isothiocyanatophenethylamine
4APA:	4-aminophenylalanine
DTPA:	Diethylene-triamine-pentaacetic acid
TTHA:	Triethylene-tetramine-hexaacetic acid
CAD:	Cadaverine or 1,5-diaminopentane

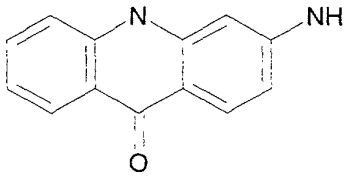
More particularly preferred compounds of the present invention include the DTPA chelates below:



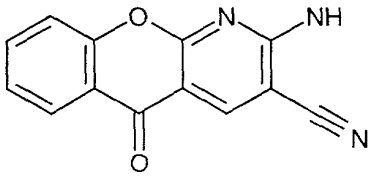
7-amino-1-azaxanth-5-one
(7AAX)



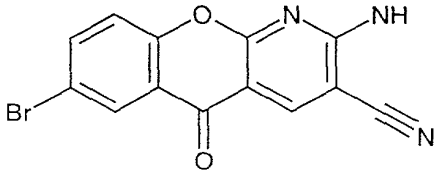
2-amino-xanthone
(2AX)

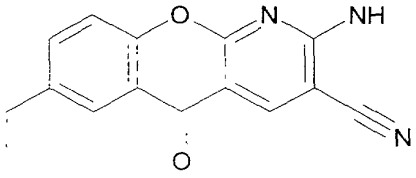


3-amino-acridone
(3AAC)



2-amino-3-cyano-azaxanthone
(2ACAX)





2-amino-3-cyano-7-bromo-azaxanthone (2ACBAX)

2-amino-3-cyano-7-ethyl-azaxanthone (2ACEAX)

Definitions

Sensitizer and chelator moiety abbreviations are as defined in Table II above.

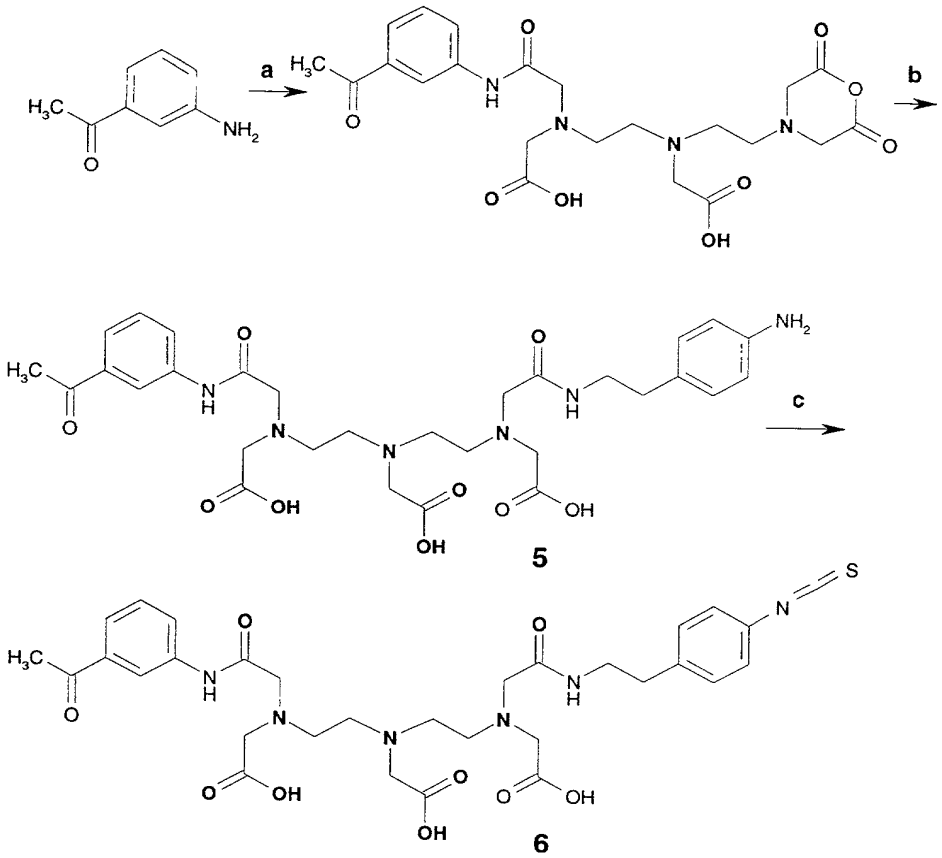
The terms "bioconjugate" and "bioconjugatable" mean the ability of a functional group or groups on a chemical moiety to form covalent linkage to biomolecules.

The term "polycarboxylate derivative of DTPA or TTHA" means a compound which differs from DTPA and TTHA by changing the length of N-acetic acid units, or by rearranging the units from a linear to a cyclic form.

The term "bioassay" means immunoassays, DNA hybridization assays, receptor binding assays, enzyme assays, cell-based assays, immunocytochemcial or immunohistochemical assays and the like.

Method of Preparation

The sensitizers and space linkers with structures described herein above are employed in a manner shown in Scheme I and in the Examples. The first step in the synthetic route involves reacting the sensitizer amine, hereby exemplified by 3-aminoacetophenone, with equal or higher molar ratio of DTPAA (diethylene-triamine-pentaacetic anhydride) in the presence of triethylamine. The product formed is not isolated but allowed to react with an equal or a slight molar excess of the linker amine, hereby exemplified by 4-aminophenethylamine. The disubstituted derivative is then isolated and purified by HPLC before converting the linker amino group into a bioconjugatable function. The final step is to react the product (Compound **5**) with thiophosgene in a slightly acidic condition to form the isothiocyanate (Compound **6**). Alternatively, a chlorotirazine derivative instead of an isothiocyanate can also be prepared from Compound **5** for facile labelling of target molecules with a reactive amino function.



a) DTPAA, DMSO, Et_3N; b) 4APEA, DMSO, Et_3N; c) $CSCl_2$, $MeCl_2$-H_2O

Utility of the Invention

The compounds of this invention can be used for labelling donor peptides, proteins, DNAs, enzyme substrates, ligand molecules in immunoassays, DNA hybridization assays, receptor binding assays, enzyme assays, cell-based assays, immunocytochemcial or immunohistochemical assays and the like. These bioassays can be also formated for ultrasensitive high-throughput screening assays. In the bioassay, the lanthanide chelate is excited in a fluorescence instrument and provide energy transfer to an acceptor molecule such as an organic dye (e.g. allophycocyanin (APC), or indodicarbocyanin or CY-5) capable of providing the desired long-lived fluorescense emission for quantitation.

The present invention also provides a method for using the compounds of Formula I in fluorescence detection-based techniques or bioassays. The present method comprises the steps of:

1. labelling an aliquot comprising donor biomolecules selected from the group consisting of: peptides, proteins, deoxyribonucleic acids (DNAs), ribonucleic acids

(RNAs), enzyme substrates, and ligand molecules with a compound of Formula I by a linking reaction with linker R2 to provide a labelled biomolecule assay sample;

2. adding a suitable amount of a suitable organic dye, preferably selected from the group consisting of: allophycocyanin (APC) and indodicarbocyanin (CY-5), to the labelled biomolecule assay sample;

3. exciting the labelled biomolecule assay sample in a suitable fluorescence instrument to provide a fluorescense emission for quantitation.

Fluorescence instruments suitable for use in the inventive method include the Photon Technology International, Model LS-100, Luminescence System.

The present invention further provides a kit for fluorescence detection-based techniques or bioassays which use the compounds of Formula I as the basis for signal detection and measurement, such kit comprising:

1. a suitable amount of a compound of Formula I; and

2. a suitable amount of organic dye, preferably selected from the group consisting of: allophycocyanin (APC), indodicarbocyanin (CY-5) and rhodamine.

Such a kit provides instructions for proper use thereof, including the appropriate amounts of the compound of Formula I and the organic dye to use for a particular bioassay sample molecular type and size.

General

Proton NMR spectra were recorded at 400 MHz using a Bruker AMX 400 spectrometer. $CDCl_3$ is deuteriochloroform, DMSO-d_6 is hexadeuteriodimethylsulfoxide, and) CD_3OD is tetradeuteriomethanol. Chemical shifts are reported in parts per million (d) downfield from the internal standard tetramethylsilane. Abbreviations for NMR data are as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets, app = apparent, br = broad. J indicates the NMR coupling constant measured in Hertz. Fourier transform infrared (FTIR) spectra were recorded on a Nicolet Impact 400 D infrared spectrometer. IR and FTIR spectra were recorded in transmission mode, and band positions are reported in inverse wavenumbers (cm^{-1}). Mass spectra were taken on either VG 70 FE, PE Syx API III, or VG ZAB HF instruments, using fast atom bombardment (FAB) or electrospray (ES) ionization techniques.

Examples

In the following synthetic examples, temperature is in degrees Centigrade (°C). Unless otherwise indicated, all of the starting materials were obtained from commercial sources. Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. These Examples are given to illustrate the invention, not to limit its scope. Reference is made to the claims for what is reserved to the inventors hereunder.

Referring to Table II and the Method of Preparation section:

Example 1

Preparation of 3AAP-DTPA (1) and 3AAP-DTPA-4APEA (5)

To a solution of DTPAA (143 mg, 0.4 mmol) in 10 mL dry DMSO and 2 mL dry triethylamine was added a solution of 3-aminoacetophenone (3AAP, 54 mg, 0.4 mmol) in 5 mL DMSO. The mixture was stirred at room temperature for 0.5 h and then treated with a solution of 4-aminophenethylamine (4APEA, 53 mg, 0.4 mmol) in 5 mL DMSO. The mixture was allowed to stir at room temperature for an additional 3 h and then evaporated to dryness. The oily residue was chromatographed on reversed-phase C18 hplc (using a step gradient of 0 to 60% acetonitrile in 0.1% TFA buffer) to give, after lyophilization, **1** as a cream colored solid and **5** as a pale yellow solid. Compound **1** was obtained in 59 mg yield. ^{1}H-NMR (CD_3OD) : d 2.60 (3H, s), 3.1-3.5 (10H, m), 3.6 (2H, s), 3.65 (2H, s), 3.71 (2H, s), 4.42 (2H, s), 7.42 (1H, dd), 7.75 (1H, dd), 7.83 (1H, dd), 8.31 (1H, d); MS: m/z 511 (M-H), Compound **5** was obtained in 16 mg yield. ^{1}H-NMR (CD3OD): d 2.62 (3H, s), 2.73 (2H, t), 3.21 (2H, t), 3.3-3.55 (12H, m), 3.65 (2H, s), 3.74 (2H, s), 4.35 (2H, s), 7.13 (4H, s), 7.41 (1H, dd), 7.75 (1H, dd), 7.83 (1H, dd), 8.32 (1H,d); MS: m/z 682 (M+ $3NH_4$), 683 (MH+ $3NH_4$).

Example 2

Preparation of 4AAP-DTPA-APEA-ITC (6).

To a solution of 4AAP-DTPA-APEA (**3**, 12 mg, 0.019 mmol) in 10 mL of 0.5 N HCl was added 4mL of thiophosgene (85% in CCl_4). The two phase reaction was allowed to stirred vigorously for 1 h . The mixture was worked up by separating the layers in a separatory funnel and the aqueos solution was washed by additional methylene chloride and then chromatographed on a small reversed-phase C18 column to give the thioisocyanate

product (**6**), an off-white solid in 10 mg yield after lyophilization. ^{1}H-NMR (CD_3OD): 2.60 (3H, s), 2.72 (2H, t), 3.20 (2H, t), 3.3-3.5 (12H, m), 3.65 (2H, s), 3.74 (2H, s), 4.34 (2H, s), 7.12 (4H, s), 7.41 (1H, ss), 7.74 (1H, dd), 7.84 (1H, dd), 8.20 (1H,d); MS: m/z 724 ($M+3NH_4$), 725 ($MH+ 3NH_4$); IR: 2108 cm^{-1} (S=C=N stretch).

Example 3

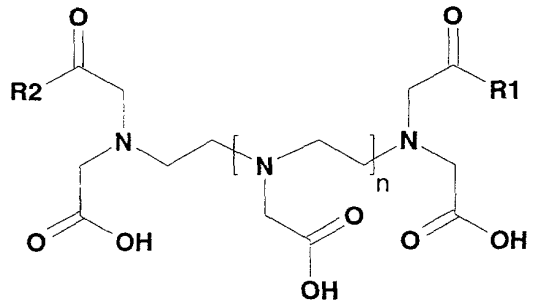
Preparation of 4ABP-DTPA (4) and 4ABP-DTPA-4APEA (12)

To a solution of DTPAA (179 mg, 0.5 mmol) in 5 mL of dry DMSO and 3 mL of dry triethylamine was added a solution of 4-aminobenzophenone (4ABP, 99 mg, 0.5 mmol) in 5 mL DMSO. The mixture was stirred for 0.5 h and treated with a solution of 4-aminophenethylamine (4APEA, 68 mg, 0.05 mmol) in 5 mL DMSO. After an additional 3 h stirring at room temperature, the mixture was evaporated to dryness. The oily residue was chromatographed on reversed-phase C18 hplc (using a step gradietn of 0-60% acetonitrile in 0.1% TFA buffer) to give **4** as a cream colored solid and **12** as a pale yellow solid. Compound **4** was obtained in 57 mg yield. ^{1}H-NMR (CD_3OD): d 3.2-3.5 (10H, m), 3.60 (2H, s), 3.63 (2H, s), 3.74 (2H, s), 4.43 (2H, s), 7.53 (2H, m), 7.62 (1H, dd), 7.76 (2H, m), 7.8 (4H, s); MS: m/z 573 (M+H). Compound **12** was obtained in 47 mg yield. ^{1}H-NMR (CD_3OD): d 2.73 (2H, t), 3.25 (2H, t), 3.3-3.5 (12H, m), 3.67 (2H, s), 3.73 (2H, s), 4.3 (2H, s), 7.23 (4H, s), 7.55 (2H, m), 7.64 (1H, dd), 7.8 (2H, m), 7.83 (4H, m); MS: m/z 691 (M+H).

The above specification and Examples fully disclose how to make and use the compounds of the present invention. However, the present invention is not limited to the particular embodiments described hereinabove, but includes all modifications thereof within the scope of the following claims. The various references to journals, patents and other publications which are cited herein comprise the state of the art and are incorporated herein by reference as though fully set forth.

We claim:

1. A compound of Formula I:



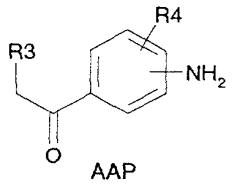
I

$[\backslash N/\backslash]_n$ is a chelator selected from the group consisting of: DTPA (n= 1), (TTHA) (n=2), and a polycarboxylate derivative of DTPA or TTHA, which chelates a lanthanide metal cation;

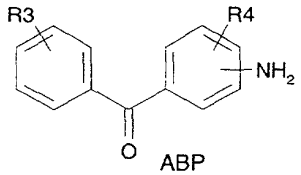
R1 is selected from the group consisting of: phenones and quinolines; and

R2 is selected from the group consisting of: OH, $NH(CH_2)_nOH$, $NH(CH_2)_nNH_2$, $NH(CH_2)_nPhNH_2$, $NH(CH_2)_nPhOH$, $NHCH(CO_2H)CH_2PhNH_2$,, $NH(CH_2)_nPhNCS$; wherein n is 1-6.

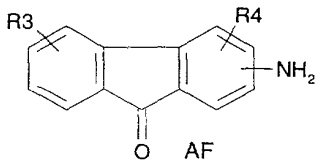
2. A compound according to Claim 1 wherein R1 is selected from the following group: aminoacetophenones (AAP), aminobenzophenones (ABP), aminofluorenones (AF), aminoxantones (AX), amino-azaxanthones (AAX), aminoanthraquinones (AAQ), aminoacridones (AAC), and aminoquinolines (AQ):



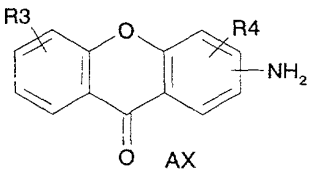
AAP



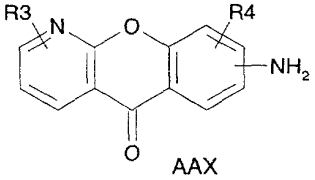
ABP



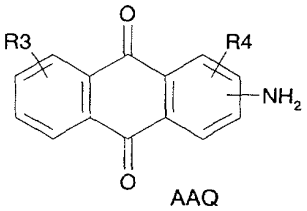
AF



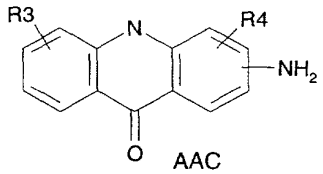
AX



AAX



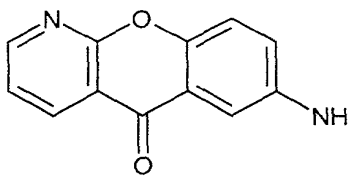
AAQ



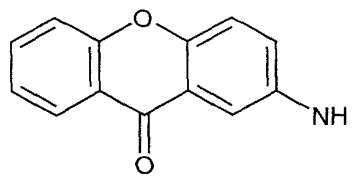
AAC

wherein R3 and R4 are independently selected from the group consisting of: H, OH, NH_2, $COCH_3$, COPh, OPh, NHPh, CN, NO_2, CO_2H, and CO_2CH_3.

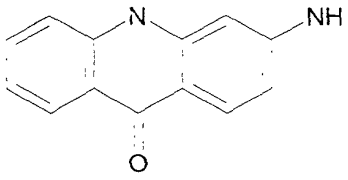
3. A compound according to Claim 1 wherein R1 is selected from the following group:



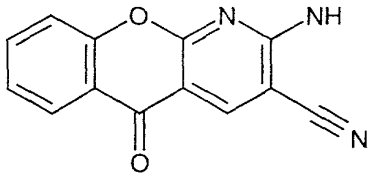
7AAX



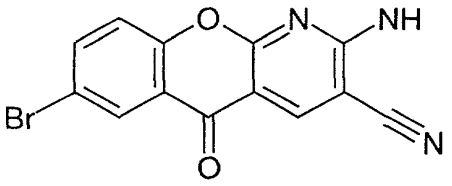
2AX



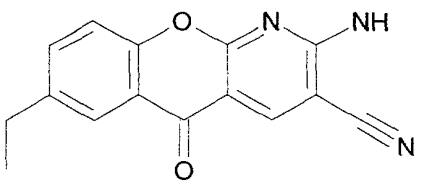
3AAC



2ACAX



2ACBAX



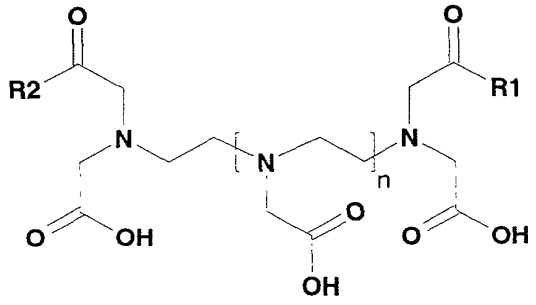
2ACEAX

4. A compound according to Claim 1 wherein $[\backslash N/\backslash]_n$ is DTPA (n= 1).

5. A compound according to Claim 1 wherein the lanthanide metal cation is selected from the group consisting of: Tb III, Eu III, Sm III, and Dy III.

6. A compound according to Claim 5 wherein the lanthanide metal cation is selected from the group consisting of: Eu III or Tb III.

7. A method for using a compound of Formula I:



I

wherein:

$[\backslash N/\backslash]_n$ is a chelator selected from the group consisting of: DTPA (n= 1), (TTHA) (n=2), and a polycarboxylate derivative of DTPA or TTHA, which chelates a lanthanide metal cation;

R1 is selected from the group consisting of: phenones and quinolines; and

R2 is selected from the group consisting of: OH, $NH(CH_2)_nOH$, $NH(CH_2)_nNH_2$, $NH(CH_2)_nPhNH_2$, $NH(CH_2)_nPhOH$, $NHCH(CO_2H)CH_2PhNH_2$,, $NH(CH_2)_nPhNCS$; wherein n is 1-6;

in fluorescence detection-based techniques or bioassays comprising the steps of:

a. labelling an aliquot comprising donor biomolecules selected from the group consisting of: peptides, proteins, deoxyribonucleic acids (DNAs), ribonucleic acids (RNAs), enzyme substrates, and ligand molecules with a compound of Formula I by a linking reaction with linker R2 to provide a labelled biomolecule assay sample;

b. adding a suitable amount of a suitable organic dye to the labelled biomolecule assay sample;

c. exciting the labelled biomolecule assay sample in a suitable fluorescence instrument to provide a fluorescense emission for quantitation.

8. A method according to Claim 7 wherein said organic dye is selected from the group consisting of but not limited to: rhodamine, allophycocyanin (APC) and indodicarbocyanin (CY-5),

9. A kit for fluorescence detection-based techniques or bioassays comprising:

a. a suitable amount of a compound of Formula I; and

b. a suitable amount of organic dye.

10. A kit according to Claim 9 wherein said organic dye is selected from the group consisting of but not limited to: rhodamine, allophycocyanin (APC) and indodicarbocyanin (CY-5).

Docket No.: P50800.

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

"NOVEL FLUORESCENT LANTHANIDE CHELATES"

the specification of which (check one)
[ ] is attached hereto.
[X] was filed on **07 July 1999** as Serial No. **PCT/US99/15366**
and was amended on (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or Inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Number	Country	Filing Date	Priority Claimed

I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below.

Application Number	Filing Date
60/091,944	**07 July 1998**

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

Serial No.	Filing Date	Status

I hereby appoint the practitioners associated with the Customer Number provided below to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and direct that all correspondence be addressed to that Customer Number:

Customer Number 20462.

Address all correspondence and telephone calls to **Yuriy P. Stercho**, SmithKline Beecham Corporation, Corporate Intellectual Property-U.S., UW2220, P.O. Box 1539, King of Prussia, Pennsylvania 19406-0939, whose telephone number is **610-270-5018**.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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